

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. V

WASHINGTON, D. C., JANUARY 24, 1916

NO. 17

A SERIOUS DISEASE IN FOREST NURSERIES CAUSED BY PERIDERMIIUM FILAMENTOSUM

By JAMES R. WEIR, *Forest Pathologist*, and ERNEST F. HUBERT, *Scientific Assistant, Investigations in Forest Pathology, Bureau of Plant Industry*

In June, 1914, several seedlings of *Pinus ponderosa* Laws., with the stems severely infected with a disease caused by a species of *Peridermium*, were received from the Savenac nursery of the United States Forest Service, at Haugan, Mont. The seedlings were taken from the field-planting area located near the nursery. They had remained one year in the seed beds, one year in the transplant beds, and two years in the field. It seemed likely that the seedlings became infected while in the nursery, since the few yellow pines in the near vicinity of the area were free from the fungus.

On July 2, 1914, *Castilleja miniata* Dougl., growing in abundance on the nursery site, was found bearing the fungus *Cronartium coleosporioides* (D. and H.) Arthur.¹ No other species of *Cronartium* was found. Evidence of the æcial stage on left-over yellow-pine seedlings in the transplant beds brought the two stages in such close proximity it seemed certain that the fungus on the pine seedlings could be no other than *Peridermium filamentosum* Peck. Since the Savenac nursery has an annual output of 1,600,000 yellow-pine seedlings, it was evident that measures should be employed immediately to prevent the spread of the disease.

On May 1, 1915, all of the 2-year-old yellow-pine seedling beds were found to be infected with the fungus. The seedlings were being prepared for shipment to the planting areas in the forests, and a thorough inspection was made of all the bundled stock. All visibly infected seedlings were removed and burned. The seedlings remaining in the beds were examined, and the infected ones similarly destroyed. More than 4 per cent of the plants gave outward evidence of being attacked. Of the 10,000 seedlings inspected 432 were removed and burned. Control

¹ Meinecke, E. P. Notes on *Cronartium coleosporioides* Arthur and *Cronartium filamentosum*. In *Phytopathology*, v. 3, no. 3, p. 167-168. 1913.

methods were devised and recommended, and, as the bundling of seedlings progressed, all visibly infected trees were removed and burned. A sharp watch was kept on the beds to remove new infections as they developed.

Most of the infections were found along the north and east borders of the seedling beds. A large patch of *Castilleja miniata* was growing on the edge of a lodgepole pine (*Pinus murrayana* "Oreg. Com.") stand near the creek bank directly northeast of the infected seedling beds and not more than 200 feet distant. The records of the weather station located on the grounds show that the prevailing winds blow both northeast and southwest, which is an important factor in spore distribution between the two hosts. Thus, these winds sweep northeast over the patch of *Castilleja miniata* from the 2-year-old yellow-pine seedlings and in reversing blow from the former to the latter. In this manner the æciospores from the infected yellow pine are distributed to the castilleja plants and the sporidia borne on the castilleja leaves are transmitted to the young trees in the beds. On May 13, 1915, this fungus infection was found to be of serious importance on the yellow pine.

From fresh specimens of the blister rust brought in to the greenhouse at Missoula, Mont., two plants of *Castilleja miniata* were inoculated on May 3, 1915. These were covered with oiled-paper bags and labeled. Six control plants of the same species were potted and bagged and kept in a separate part of the greenhouse. On May 23 uredospores developed on the underside of the leaves of the two inoculated plants, while the control plants remained normal. Later the teliospores developed, sporidia being produced on May 29. Duplicate experiments were conducted at the field camp at Priest River, Idaho. Æciospores from the infected yellow-pine seedlings were sown on *Castilleja miniata* on May 14, and they gave positive results on June 11. The characteristic filamentous structure of the æcia on the pine seedlings and these transfers of the fungus to castilleja prove the fungus to be *Peridermium filamentosum* Peck. •

On May 13, 1915, the native lodgepole pine surrounding the nursery was found to be infected with a trunk, a branch, and a needle form of *Peridermium*. The structure of the æcia of these forms indicated that the trunk and the branch forms were identical. The trunk form (known locally as the "hip canker" of the lodgepole pine) and the branch-gall form in the Rocky Mountain region have been commonly united under the name "*Peridermium harknessii* Moore."¹ Later they were transferred to *Peridermium cerebrum* Peck by Arthur and Kern.²

The following inoculations, made recently at Missoula, Mont., by the writers, prove that the "hip canker" and the gall-forming *Peridermium* of the lodgepole pine are both *Peridermium filamentosum*.

¹ Harkness, H. W. New species of California fungi. In *Bul. Cal. Acad. Sci.*, v. 1, no. 1, p. 37, 1924.

² Arthur, J. C., and Kern, F. D. North American species of *Peridermium* on pine. In *Mycologia*, v. 6, no. 3, p. 133-138, 1914.

On May 17, 1915, æciospores from the "hip canker" of *Pinus contorta* from Haugan, Mont., were sown on two plants of *Castilleja miniata* under control conditions in the greenhouse at Missoula. On June 3 uredospores were present on the leaves. The teliospores appeared June 14. The two control plants remained healthy. The Cronartium was identical with that previously produced by the inoculations on *Castilleja miniata* with æciospores from the *Peridermium* on the 2-year-old seedlings of *Pinus ponderosa*. This demonstrates the identity of the "hip canker" *Peridermium* with *Peridermium filamentosum*.

The following cultural data show that the gall-forming *Peridermium* of the lodgepole pine is likewise identical with *Peridermium filamentosum*. On May 25, 1915, æciospores from the gall-forming *Peridermium* on branches of lodgepole pine were sown by the writers on three plants of *Castilleja miniata* under control conditions in the greenhouse. By June 11, 1915, uredospores had developed on the leaves, telia and sporidia being produced 10 days later. The two control plants remained healthy.

Check experiments carried on at the field camp at Priest River, Idaho, gave similar positive results. Six plants of *Castilleja miniata* were inoculated and gave positive results. All three control plants remained healthy.

Cultures, under control, made both in the greenhouse and in the field, on *Castilleja miniata* with æciospores taken from the blister rust on the lodgepole pine commonly known as *Peridermium stalactiforme* A. and K., have produced *Cronartium coleosporioides* (D. and H.) Arthur. Two plants of *Castilleja miniata* were inoculated and two control plants set aside. Both inoculated and control plants were covered with oiled-paper bags. The inoculated plants gave positive results and the controls remained healthy. This confirms the results of Meinecke¹ and the conclusions of Arthur and Kern² and places *Peridermium stalactiforme* without further doubt under *Peridermium filamentosum*.

The absence of oaks (*Quercus* spp.), the alternate hosts of *Peridermium harknessii*³ and *Peridermium cerebrum*, from this region where the species of *Peridermium* on the lodgepole pine is so prolific, the characteristic filamentous processes in the æcia of the various forms of *Peridermium* appearing on the lodgepole pine, and the inoculation experiments successfully conducted on *Castilleja miniata*, all exclude the possibility of this fungus being other than *Peridermium filamentosum*.

The yellow-pine seedlings in the nursery were free from traumatic injuries. This is explained by the fact that they had remained in the same bed since germination and thus were not exposed to the injury from transplanting. All seedlings showing slight corrugations or blisterings of the lower stems gave no evidence of mechanical injury, but they

¹ Meinecke, E. P. Op. cit.

² Arthur, J. C., and Kern, F. D. Op. cit.

³ Hedgcock, G. G. Notes on some western Urediniæ which attack forest trees. II. In *Phytopathology*, v. 3, no. 1, p. 15-17. 1913.

developed the bright orange eruptions of the rust later. It is safe to draw the conclusion that the spore tubes which produce the infections in the seedlings penetrate the host in the absence of all surface openings due to the mechanical injuries. The period of development between the time of penetration of the host and the appearance of the æcial eruptions on the stems is about 10 to 11 months. The seedlings in question were produced from seed sown in the spring of 1913, and the spring of 1914 some of the seedlings produced the æcial eruptions. The seedlings must have been infected in the period following germination and have developed the fruiting stage in the spring of the following year. The infecting spores could have been either sporidia from the species of *Cronartium* on *Castilleja miniata* or possibly æciospores from the surrounding lodgepole pines infected with *Peridermium filamentosum*. Facultative autoecism in *Peridermium filamentosum* is as yet not proved, but it is suspected of being a "repeater."

During the period from May 29 to June 2, 1915, Mr. E. C. Rogers, of the Forest Service nursery at Haugan, Mont., assisted in the work of visiting and inspecting the various plantation areas near Wallace, Idaho, on the Coeur d'Alene National Forest, and those in the vicinity of Savenac nursery and Deborgia, Mont., on the Lolo National Forest. In all an area of approximately 500 acres was covered. The inspection was confined principally to the yellow-pine plots, with particular attention to the plants taken from the infected 2-year-old yellow-pine beds at Savenac nursery. Very few infections caused by species of *Peridermium* were recorded, some of the areas being entirely free from visible signs of the rust, although it may be present and not appear until the following year or later. In the case of the "2-year-old yellow pine, unfertilized" plot, which was planted in the spring of 1915, the few infections observed were found to be covered by the moist earth because of deep planting and thus were rendered practically incapable of spreading. Two of these infections were molded and the spores were no longer viable. The Placer Creek area near Wallace, Idaho, is a clean-burn site, the fires of 1910 having destroyed all living timber. No living pines or *castilleja* plants are to be found growing within a considerable distance of this area. *Castilleja miniata* and *Pinus contorta* are plentiful in the area containing 4-year-old yellow-pine seedlings located on the ridge west of the Savenac nursery. Very little visible infection was found on this plot. These facts prove the effectiveness of the inspection work in checking the spread of the disease and the necessity for culling out and burning the infected seedlings as soon as the eruptions make their appearance.

On June 1, 1915, a survey was made of the area surrounding the nursery beds for a distance of half a mile. Fifty per cent of the lodgepole-pine stand in close proximity to the beds was badly infected with *Peridermium filamentosum*. A group of 61 trees, having diameters (breast

high) of 5 inches and over and growing within 100 feet of the nursery beds, was found to be very seriously infected. Of the 61 trees, 26 had large cankers encircling the trunks varying in length from 2 to 8 feet. The branches and twigs were infected. *Peridermium montanum* was also present on the needles. *Castilleja miniata* was found growing in abundance under the trees. Lodgepole-pine seedlings in and near this area were, with rare exceptions, heavily infected with the twig and stem and the needle forms of *Peridermium*. Very little native yellow pine was found growing in the vicinity, most of the trees having been killed by the fires of 1910. A few veteran trees remain growing upon the ridge west of the nursery, but these show no evidence of fresh eruptions of *Peridermium*. These facts point to the lodgepole pine as the original distributor of infection to the yellow-pine seedling beds in the nursery.

Experiments are being conducted in an effort to control the disease. The seedlings in the nursery beds are being sprayed during the infection period. An effort is being made to eradicate the alternate host from the vicinity by mechanical or chemical means. The felling and burning of trees near by infected with *Peridermium* will reduce the chances of infection. The possibility of the fungus possessing facultative autoecism, the close proximity and abundance of the alternate host, and the prolific development of the same fungus upon lodgepole pine in the vicinity of the seedling beds all make *Peridermium filamentosum* a dangerous enemy to deal with in this nursery and one to be reckoned with in other forest nurseries where similar conditions exist.

SUMMARY

Peridermium filamentosum Peck has been found to cause a serious disease of yellow-pine seedlings at the Savenac nursery located at Haugan, Mont.

The various forms of *Peridermium* occurring on lodgepole pine at this nursery, with the exception of the foliicolous species, have been demonstrated to be *Peridermium filamentosum*, having an alternate stage on species of *Castilleja*.

The fact that the same species of *Peridermium* attacks both the lodgepole pine and the yellow pine increases the difficulty of control of this fungus.

The proximity and abundance of the alternate host (*Castilleja miniata*) of *Peridermium filamentosum* and its prolific development on lodgepole pine in the vicinity of the seedling beds tend to make this disease a dangerous one in forest nurseries.

SWEET-POTATO SCURF

By L. L. HARTER,

Pathologist, Office of Cotton and Truck Disease Investigations,
Bureau of Plant Industry

INTRODUCTION

The scurf disease of the sweet potato (*Ipomoea batatas*) was first described by Halsted,¹ who published a brief account of it in 1890. To the fungus he gave the name *Monilochaetes infuscans*, a new genus and species, of which, unfortunately, he gave no technical description. For many years following his pioneer work little or no attention was given to sweet-potato diseases. This very common and interesting disease was therefore passed over until a few years ago, when the writer and others took up a study of them. For almost five years the disease has been under observation and study. It is therefore for the purpose of completing the description of the organism and recording the results of inoculation experiments and certain characteristics of the fungus heretofore unpublished that this paper is prepared.

GENERAL APPEARANCE OF THE DISEASE

Scurf is characterized by a brown discoloration of the surface of the underground parts of the sweet potato (Pl. LVII). The discolored areas may occur as spots of varying size and shape, with no definite outline, or as a uniform rusting of the entire surface. In gross appearance it reminds one somewhat of the silver scurf of the Irish potato, although it is somewhat darker. However, it does not penetrate the host to the extent that silver scurf does. The scurf of the sweet potato produces no rupture of the epidermis and is so superficial as to be easily scraped off by the finger nail.

DISTRIBUTION, PREVALENCE, AND LOSS

The writer has found the scurf very prevalent on sweet potatoes in New Jersey, Delaware, Maryland, Virginia, North Carolina, Ohio, Illinois, Iowa, and Kansas, and to a slight extent in other States. The following varieties are susceptible to scurf in varying degrees: Eclipse Sugar Yam, General Grant Vineless, Florida, Nancy Hall, Yellow Yam, Miles Yam, Red Brazilian, Dahomey, Yellow Strasburg, Pierson, Key West Yam, Vineless Yam, Southern Queen, Big Stem Jersey, Yellow Jersey, and Early Carolina. It is probable that the disease occurs on other varieties as well.

¹ Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Exp. Sta. Bul. 76, p. 25-27, fig. 17, 1890.

Scurf is more prevalent in heavy, black soils and in soils that have been heavily manured or contain a larger amount of organic matter than in light, sandy soils.

The loss to the crop caused by the scurf is perhaps small in comparison with that caused by some of the more virulent diseases. Nevertheless, the actual financial loss throughout the country that can be attributed to this disease alone amounts to considerable. Scurfy potatoes do not command as high a price in the markets as clean ones, though if otherwise sound they are just as good for consumption. The fungus under favorable conditions, such as a relatively high humidity and temperature, continues to develop under storage conditions to a limited degree. It weakens the host, so that during periods when the storage house is rather dry the potato loses moisture and becomes shriveled and dried, rendering it unfit for sale and at the same time less resistant to the attacks of other parasites. Taubenhaus¹ claims that the fungus on the potato is easily killed by immersing for 10 minutes in a solution of mercuric chlorid (1:1,000).

ISOLATION OF THE FUNGUS

Some difficulty was experienced at first in isolating the fungus, since it proved to be a very slow grower and developed but little or not at all on some kinds of media. After some experimentation with different media it was found to make a slow growth in Irish-potato, string-bean, and oatmeal agar. By thoroughly washing the potato and disinfecting for about one minute in a solution of mercuric chlorid (1:1,000) and planting bits of the tissue in plates of oatmeal agar by means of sterile instruments a pure culture could generally be secured. In a week or 10 days transfers were made to media in test tubes, usually cooked rice in water or sterile, moistened corn meal. At the end of three or four weeks on these media a matted growth of dark-brown hyphæ developed. Hyaline spores are produced in abundance on long, stout conidiophores in tubes of cooked rice.

INOCULATION EXPERIMENTS

Inoculation experiments were begun on October 13, 1914, and performed as follows: Sound potatoes were thoroughly washed in water and placed in moist chambers with moistened filter paper in the bottom. They were then sprayed with a suspension of spores and bits of broken hyphæ of the scurf fungus in sterile water and exposed to laboratory room conditions. Water was added from time to time, as necessity required, to maintain the humidity of the moist chamber. At the end of two weeks small centers of infection appeared indiscriminately over the surface of the potatoes. These centers gradually enlarged, either by the merging of two or more spots or by the enlargement from a single center. There is undoubtedly considerable enlarging of the spots in moist chambers from

¹ Taubenhaus, J. J. Soil stain and pox, two little known diseases of the sweet potato. (Abstract.) *The Phytopathology*, v. 4, no. 6, p. 405. 1914.

centers of infection, in view of the fact that conidiophores often 200μ in length stand erect or at an angle on the surface of the potato and drop their spores, starting new infections outside the point of original growth. The spots, however, so far as the writer has been able to determine, do not enlarge by the branching and creeping of the hyphæ over the surface. Repeated inoculation experiments gave similar results. The checks remained free from the disease.

DESCRIPTION OF THE FUNGUS

The young vegetative growth of *Monilochaetes infuscans* is hyaline and septate. At the end of a few days, however, with the exception of the terminal cell of the conidiophore, the hyphæ turn densely brown. On the host little or no branching of the vegetative growth takes place. Although Halsted figured a branching of the hyphæ which was hyaline in color within the tissues of the host, the writer, after long and detailed examination of paraffine sections and sections prepared in other ways, has not been able to find a sure example. The sporophores, for such they appear to be, arise from the surface of the host and are attached to it by an enlarged end cell slightly buried in the cuticle (Pl. LVIII, E, C, D). Occasionally a second (Pl. LVIII, I) or third (Pl. LVIII, J) enlargement or bulblike growth is found deeper in the host or parallel with the surface (Pl. LVIII, G). From some of these secondary enlargements a conidiophore may be developed (Pl. LVIII, F, H). Plate LVIII, E, C, shows conidiophores bearing conidia produced on the host. The brown septate conidiophores vary in length from 40 to 175μ and bear at the end a single-celled spore, which on the host is slightly brown or hyaline. The conidia are 12 to 20μ in length by 4 to 7μ in thickness.

This fungus, as might be expected, behaves differently when grown artificially. Growth has been carefully observed on a few of the common media—namely, Irish-potato agar, beef agar, rice agar, oatmeal agar, string-bean agar, Irish-potato cylinders, sweet-potato stems, and stems of *Melilotus alba*. At the end of 24 days a very slight growth appeared on string-bean agar, rice agar, and oatmeal agar at a temperature varying from 6° to 7° C. Conidia were very sparingly produced. At room temperature (23° to 26°) growth was visible on all media in 4 days, except on rice agar and the stems of sweet potatoes and *Melilotus alba*. In 13 days a small growth appeared on rice agar, but on stems of sweet potatoes and sweet clover no growth was detected at the end of 4 weeks. There is very little difference in the gross appearance of the growth on any of the media used. Enlargement from a single center is very slow, attaining a diameter of about 2 to 5 mm. in 14 days. The fungus piles up in an almost black feltlike mass 2 to 3 mm. in height, with an entire margin. It penetrates the medium but little. The vegetative hyphæ in mass are almost charcoal-black, although in gross appearance there is some variation on different culture media. On Irish-

potato cylinders and Irish-potato agar the growth has a darker appearance than on oatmeal agar, beef agar, and string-bean agar, owing to the fact that the numerous erect conidiophores bearing hyaline spores are produced in greater abundance on the three latter media and give a grayish appearance to the upper surface. If the conidiophores and spores be scraped away, the mass is black beneath. Growth appeared only on oatmeal agar at temperatures varying from 30° to 32° in 14 days. From these results it appears that temperatures as low as 6° to 7° and as high as 30° to 32° prohibit the normal growth of the fungus.

The vegetative growth on artificial cultures is hyaline at first and later brown (Pl. LVIII, L), with the exception of the end cell of the conidiophore, which at its outer extremity is hyaline to slightly brown (Pl. LVIII, A, B, L). The conidiophores are branched, septate (Pl. LVIII, A, L), and vary in length from 30 to 225 μ . The conidia are continuous, granular, and hyaline to slightly brown with age (Pl. LVIII, M). As soon as one conidium is mature, it separates easily from the conidiophore and another begins growth by a swelling of the end cell of the conidiophore, to be dropped in turn when mature. This process is repeated as long as the environment of the host will permit. It should be noted in this connection also that this fungus can be reproduced by hyphae as well as from the spores. It is likely also that vegetative reproduction accounts for a larger part of the infections under natural conditions. In fact, certain vegetative parts might be confused with or mistaken for conidia. Although conidia are not produced in abundance on the host, they frequently develop normally on diseased potatoes kept for some days in a moist chamber.

The conidia under laboratory conditions germinate slowly in rice or sweet-potato decoction. One or two growths (Pl. LVIII, K) are thrown out usually at the end of the conidia, which attain in 24 hours a length about equal to that of the spore. The branching of the hyphae begins the second day (Pl. LVIII, N), and the production of the brown pigment in about three days.

TAXONOMY OF THE FUNGUS

Halstead attributed the scurf to a new genus and species, *Monilochaetes infuscans*, but he gave no technical description of it that the writer has been able to find. The fungus belongs to the Dematiaceae of the Hyphomycetes. However, the writer has been unable, after considerable study of the fungus, to fit it into any of the genera so far described. It is, however, desirable, in view of the fact that it is a rather common and conspicuous fungus, that it have a description by which it may be recognized. The fungus has been known as *Monilochaetes infuscans* and as the cause of the sweet-potato scurf for 25 years. Taubenhaus and Manns¹ in a recent publication likewise refer to *Monilochaetes infuscans*

¹ Taubenhaus, J. J., and Manns, T. F. The diseases of the sweet potato and their control. Del. Agr. Exp. Sta. Bul. 109, p. 11. 1915.

as the cause of the disease. In view of these facts, it is believed preferable to give it a description and permit it to maintain generic rank rather than to place it in a genus where it does not naturally belong.¹

MONILOCHAETES

Hyphae dark, erect, rigid, septate, not in definite fascicles; conidia distinctly different from the sporophores and hyphae, hyaline, slightly brown with age, continuous, not in chains, acrogenous.

Monilochaetes infuscans

On the host definite vegetative hyphae are lacking; sporophores septate, erect, unbranched, dark, and attached to the host singly or by twos, by a bulblike enlargement 40 to 175 μ long, 4 to 6 μ wide, bearing rarely a hyaline one-celled oblong spore. In cooked rice the hyphae are much branched, septate, brown; sporophores brown except at terminal cell, which is frequently hyaline to slightly brown, septate, branched, stout, 30 to 225 by 4 to 6 μ ; conidia abundant, one-celled, hyaline, ovoid to oblong, 12 to 20 by 4 to 7 μ , solitary, terminal.

Parasitic on the underground parts of *Ipomoea batatas*. Type specimens deposited in the pathological collection of the herbarium of the United States Department of Agriculture, Washington, D. C.

SUMMARY

The scurf disease of the sweet potato was first recognized in 1890 by Halsted, who named the fungus "*Monilochaetes infuscans*," a new genus and species. He failed, however, to describe either the genus or species. The scurf has been found prevalent in nine States and sparingly in others, and on 16 varieties of sweet potatoes. The organism has been shown by inoculation experiments to be the true cause of the disease. A detailed discussion of the morphology of the organism is taken up, also its growth on different culture media at different temperatures. It was found that the organism on the host consisted merely of sporophores and conidia. In culture, however, well-defined branched mycelia and spores developed.

¹ The writer is indebted to Dr. C. L. Shear and Mrs. Flora W. Patterson, of the Bureau of Plant Industry, for having examined specimens of this fungus.

PLATE LVII

A sweet potato showing the discoloration produced by *Monilochaetes infusans*.

(792)



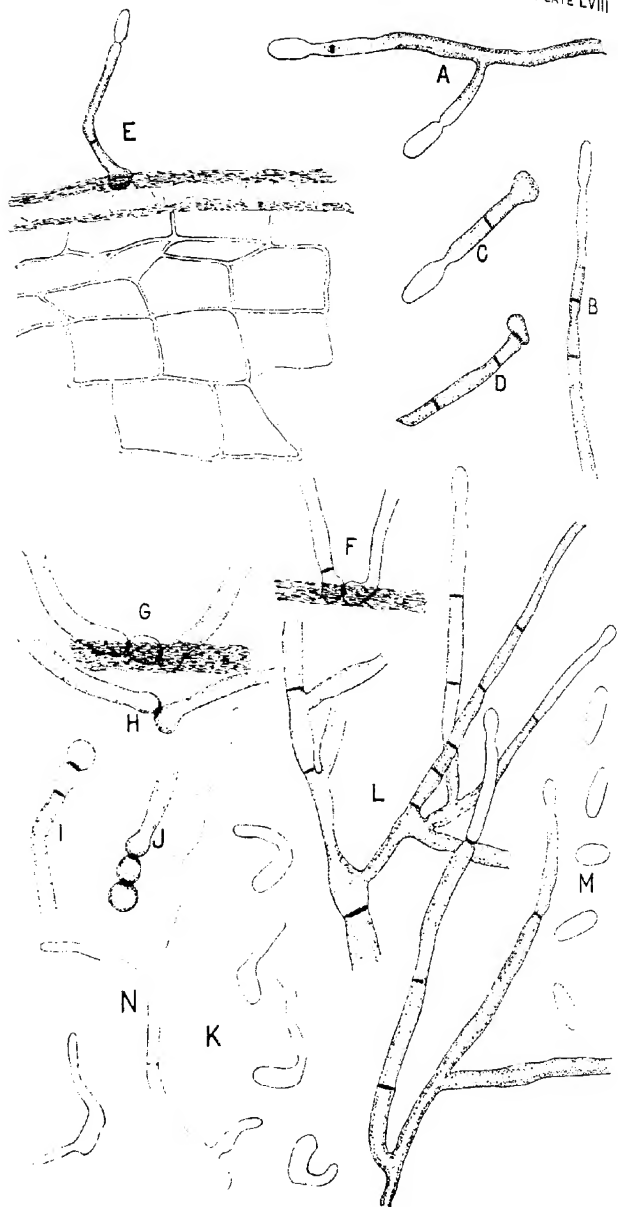


PLATE LVIII

Monilochaetes infuscans:

A, a branched conidiophore with conidia attached. *B*, an unbranched conidiophore, showing septation; conidium attached. *C*, a conidiophore from host, with conidium attached. *D*, a conidiophore from the host, showing the peculiar basal cell and septation. *E*, a conidiophore bearing conidium, showing diagrammatically the attachment to the host by a bulblike enlargement of the basal cell. *F*, two conidiophores joined at the base and slightly sunken in the tissue of the host. *G*, two conidiophores joined by a single oblong cell. *H*, two conidiophores joined at the base and slightly sunken in the tissue of the host. *I*, a conidiophore from the host with an almost spherical cell attached to the enlarged end cell. *J*, a conidiophore, showing an attachment of two almost round cells to the enlarged basal cell. *K*, germination and growth of conidia in a sweet-potato decoction in 24 hours. *L*, hyphæ from a culture, showing characteristic branching and septation. *M*, a group of mature conidia. *N*, germination, growth, branching, and septation of the fungus at the end of 42 hours in a sweet-potato decoction.

E is drawn to a scale of 200; all others to a scale of 500.

BANANA AS A HOST FRUIT OF THE MEDITERRANEAN FRUIT FLY

By E. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant*,
Mediterranean Fruit-Fly Investigations, Bureau of Entomology

INTRODUCTION

The banana export trade of the Hawaiian Islands amounted to 256,319 bunches of Chinese bananas (*Musa cavendishii*) during the year ending June 30, 1915. Although 25,448 bunches were shipped during June, 1915, the monthly average for the year was 19,621. With such a trade with the California coast established, it became imperative to determine to what extent bananas are infested by the Mediterranean fruit fly (*Ceratithis capitata* Wied.), in order that data might be placed on file for the guidance of the Federal Horticultural Board in forming its quarantine regulations for the protection of mainland fruit interests. While it has been proved that bananas may serve as host fruits of this fruit fly when ripe, all data happily corroborate the general belief among shippers and growers, as well as among entomologists familiar with the situation, that Chinese bananas and Jamaica or Bluefield bananas (*Musa* spp.), when cut and shipped under commercial conditions, are immune to attack and offer no danger as carriers of this pest if properly inspected and certified as provided for by the regulations of the Federal Horticultural Board (8).¹ These regulations, it may be stated, provide for inspection in the packing sheds for the presence of prematurely ripe, bruised, cracked, and decayed fruits; require the use of safe packing material; and prohibit the shipment of bananas from plantations the surroundings of which have not been favorably passed upon from a fruit-fly standpoint by a representative of the Board.

EVIDENCE FROM TRAPS AS TO THE PRESENCE OF ADULT FRUIT FLIES IN BANANA PLANTATIONS

The establishment of a series of traps among banana plants has shown that adult fruit flies are everywhere present in banana plantations in Hawaii. Traps were placed in the Moanalua, Moiliili, Waikiki, Mokuleia, Kawaihapai, and Puuiki plantations. As many as 793 adult flies were taken in one trap suspended from a bunch of bananas in a field at Moanalua between July 28 and August 7, 1913. Traps hung in the much larger and exceptionally well isolated banana fields of Puuiki, Kawaihapai, and Mokuleia in the Waialua district of Oahu showed a

¹ Numbers in parentheses refer to "Literature cited," p. 503.

far smaller number of adults, yet a sufficient number to infest bananas were they readily subject to infestation. In this district 57 traps caught no flies between August 9 and 21, 1913, while the average for the same period for 119 traps in which flies were caught amounted to 7.5 adults. Flies were taken in all traps hung at Moanalua, Waikiki, and Moiliili, although some of the traps were hung in the center of the largest blocks of trees. At Moanalua as few as 22 and as many as 3,334 adult flies were taken from individual traps between July 15 and August 29, 1913, while at Waikiki and Moiliili as few as 1 and as many as 402 adults were taken between June 17 and July 8, 1913. Thirty-six was the largest number of flies taken from any trap at Waialua between August 9 and 21, 1913. Although only males were caught in the traps, adults caught in the hand net showed the sexes to be present in the usual proportion among the banana plants. These data determine at once the fact that the general immunity of bananas is not due to any lack among banana plants of adult fruit flies capable of ovipositing.

ABSENCE OF INFESTATION AMONG RIPE AND GREEN BANANAS, AS EVIDENCED BY FIELD INSPECTIONS AND LABORATORY REARINGS

During the period of somewhat over three years that the Federal Government has had supervision of the inspection of export bananas in the Hawaiian Islands (from August, 1912, to the present time) the writers have seen no case of infestation among ripe or green bananas grown under normal field conditions, and neither have the banana inspectors. Frequently individual fruits on a bunch of bananas will ripen in advance of the other fruits. When the bunches are cut, these prematurely ripe fruits, which often in addition have the peel split so as to expose the pulp, are removed before shipment and discarded at the packing sheds. If any bananas are subject to infestation, it would seem that these fruits are most likely to be; yet 1,044 prematurely ripened fruits brought to the laboratory during 1913 and 1914 and placed in rearing jars yielded no adult flies, although they came from fields known to harbor adult flies. During August, 1914, when large numbers of flies were maturing from peaches in a garden in Manoa Valley, fully ripe Chinese bananas, and a variety known to the Hawaiians as the apple-banana (*Musa* sp.), growing in the midst of other species of infested fruits, showed no infestation. Thirty-nine fully ripe apple-bananas grown near the insectary from which flies were continually emerging showed no infestation. An examination of 27,000 fruits of the Chinese banana ready for shipment at several banana fields at Moanalua during early July, 1913, when records showed the adult flies to be very abundant, failed to reveal a single distinct egg puncture. Even suspicious abrasions were investigated and found not to extend through the skin nor to contain fruit-fly eggs. An examination of 3,500 similar fruits at Kalauao during July, 1913, also gave negative results. No fruit flies have been reared from about 1,000 green Chinese bananas

discarded at time of shipment at the packing sheds because of split peelings or black decayed ends. Fifty fruits of the Hawaiian variety, known as the "ice-cream" banana (*Musa* sp.), cut from the tree as they were turning color, showed no infestation, though growing in the midst of other species of infested fruits. No infestation was found among 500 overripe fruits of the Manila Hemp banana (*Musa textilis*) growing near the corner of King and Punahon Streets, Honolulu, nor among 60 fruits of the Borabora banana (*Musa fehi*), known to the Hawaiians as the Polapola banana, in a ripe though not soft condition, growing in a mountainous ravine at the head of Manoa Valley, Oahu.

There are no records of infestation of the Chinese and Bluefield bananas grown under commercial conditions in the Hawaiian Islands, or developing and ripening in city lots.

INFESTATION OF POPOULU AND MOA VARIETIES

The only case of infestation among bananas growing in the field was brought to the attention of Mr. David Haughs, of the Territorial Board of Agriculture and Forestry, on October 17, 1913. The infested fruits were of the Popoulu and Moa varieties (2) of the Popoulu group (*Musa* spp.) of cooking bananas. These are short, thick bananas, with comparatively thin skins. They are never eaten raw and, unlike the Chinese or Bluefield bananas, are rarely, if ever, shipped from the islands. They are very scarce and are strikingly distinct both from the ordinary cooking banana and from the banana of commerce.

Of the 11 fruits on the bunch of Popoulu bananas when the examination was made 7 were still green, though on the point of turning yellow, and 4 had turned yellow. There were in the peel no splits nor mechanical injuries and there was every evidence that the punctures found in three of the four ripe fruits had been made while the bunch was still on the tree. Mr. J. C. Bridwell, of the Hawaiian Board of Agriculture, had charge of the rearing, but kept no definite record of the number of adult flies reared from infested fruits. That larvæ matured and emerged from one fruit at least is evidenced by the numerous emergence holes in the peel (Pl. LIX, fig. 1).

The Moa variety was growing in the same garden with the Popoulu banana. The fruits of this variety are much larger and the peel thicker. Of 9 fruits taken from the single bunch found, 5 were perfect, but the peel of the 4 other fruits was so cracked that the pulp was well exposed; all were green in color but mature and about to turn yellow. Mr. Bridwell's notes, which have been placed at the writers' disposal through the courtesy of the Territorial authorities, state that of 12 distinct attempts at oviposition made in the peel of the 4 sound fruits, only one puncture was sufficiently deep to contain eggs, but no eggs were deposited. Only one of the 4 cracked fruits developed larvæ, and the eggs from which

these hatched were laid directly into the pulp along the crack in the peel. Of the punctures found in the peel of the cracked fruits, only one contained eggs, and these were dead and shriveled. Mr. Bridwell kept no definite record of the number of adult flies reared, but it was large. He estimates that from the Popoulu and the Moa fruits he reared about 330 adults. The thoroughness with which the larvæ destroyed the pulp of the Moa banana is shown in Plate LIX, figure 2.

Special attention should be called to the fact that infestation of the pulp in these two varieties occurred only in the fully ripe and yellow fruits of the Popoulu variety, which has a very thin skin, and in the fruits of the Moa variety, the peel of which was cracked, thus removing from the exposed pulp beneath the natural barrier to infestation referred to below. The ordinary cooking bananas, such as are in general use in the islands, are quite unlike the Popoulu and Moa varieties in shape.

EXPERIMENTS TO FORCE INFESTATION

While infestation of Hawaiian bananas has never been known to occur among fruits grown and harvested in accordance with trade requirements and prepared for shipment in accordance with the regulations of the Federal Horticultural Board, experiments have been carried on under more or less artificial and abnormal conditions for the purpose of determining whether the general immunity of commercially grown bananas in Hawaii is due to the presence of other host fruits for which the fruit fly has a greater preference or to some characteristic which renders them actually immune. Such experiments have been completed both in the field and in the laboratory.

EXPERIMENTS IN THE FIELD

As the writers have found that in the field they can bring about an infestation of ripe bananas, or in the laboratory of green but well-grown bananas that have been cut from the tree so long that the protecting sap has ceased to flow to any extent, their field experiments have been confined mainly to forcing, if possible, an infestation of bananas still attached to the tree yet sufficiently mature for the export trade.

During March, 1913, a rearing cage, 9 by 15 by 24 feet, was built over 20 Chinese banana trees bearing 14 bunches of bananas. Into this cheese-cloth-covered cage (Pl. LXII, fig. 1, 2) were introduced from time to time a total of over 3,000 Mediterranean fruit flies. The foliage within the cage was sprayed every few days with a solution of pineapple juice and water, as there was nothing else upon which the fruit flies could feed. As the fruits on the various bunches ripened, they were cut and placed in rearing jars in the insectary. The 14 bunches represented approximately 1,000 fruits, which ripened over a period extending from the middle of March to June 28. No adult flies developed from any of this fruit.

In order more closely to confine gravid females with bananas ripe enough for shipment, a fine wire cylinder, 20 inches in diameter and

30 inches long, closed at each end by cheesecloth, was placed over the entire bunch. From 200 to 500 fruit flies were introduced through the lower opening and allowed to remain with the fruit from 24 to 48 hours. The cage was then removed, the bunch cut, and the individual fruits examined for evidences of oviposition. Out of a total of 1,449 fruits thus carefully examined, 1,363 showed no evidence of attempted oviposition, while 86 bore puncture marks. In the peel of these 86 fruits the females had made 169 breaks in attempts to oviposit. Only two punctures were sufficiently deep to permit oviposition, and of these only one contained a single egg. This egg was deposited between August 21 and August 23, 1913, and by August 27, when the examination was made, fully two days after the egg should have hatched under normal conditions, it was found dead and blackened. None of the other attempts at oviposition extended for more than one thirty-second of an inch below the surface, while nearly all were mere abrasions. In all cases, however, each break in the skin was surrounded and quite well sealed by dried, sticky exudations. In a few instances the sap flowed from 1 to 2 inches down the side of the fruit from the puncture.

Before bunches of bananas are cut in the field they are stamped by the official marker of the shipper. Ten bunches stamped on June 21 were allowed to remain growing to determine whether the development that takes place during a 10-day period after the fruit is sufficiently mature for shipment lessens the general immunity it enjoys if cut when marked. It should be stated here that unless bananas are cut for shipment on the steamer for which they are marked they become too mature or, to use trade terms, too "full" or "fat," to stand without decay the 9- to 14-days' interval before they are exposed for sale in the San Francisco market. Only 9 fruits out of 505 on 4 of these 10 bunches caged with fruit flies between June 21 and June 23 bore evidences of attack, there being such evidence in 14 places. All punctures were empty, except one containing 5 eggs. These eggs had been laid in a crack caused by the decay of the blossom end of the fruit. While these eggs hatched, the larvæ immediately died. Out of 238 fruits on 2 bunches caged with fruit flies between June 23 and 26, 42 showed 159 breaks in the peel made by flies. Of these only 3 contained eggs—3, 4, and 6, respectively. An examination of these eggs on July 7 showed that while they had hatched, the larvæ were not able to mature and had died in the punctures. There were 126 attempts at oviposition in 46 out of 202 fruits on 2 bunches caged with fruit flies between June 26 and June 28; of these punctures only 2 contained eggs—1 and 3, respectively. While 3 of these eggs hatched, the larvæ died without entering the pulp. No eggs were found in 26 punctures in the peel of 15 out of 200 fruits on the last 2 bunches of those marked "June 21," and caged with fruit flies between June 28 and June 30. Plate LXI, figure 2, is reproduced from a photograph of the blossom end of a Chinese banana taken 16 days after it was

marked for shipment. The 18 punctures found on this fruit were made between June 28 and 30, or 7 to 9 days after the fruit was marked for shipment. All of these punctures were empty, and only 2 were sufficiently deep to contain eggs. The dried exudations have been removed.

Having failed to force Mediterranean fruit flies to oviposit successfully in the field in bananas sufficiently mature for the export trade, freshly laid eggs were removed from apples and placed in incisions made in the peel of bananas marked for shipment but still attached to the tree. Small cuts varying from one-fourth to one-half inch in length, extending with the grain of the peel but not quite reaching the pulp, were made. From these cuts the sap flowed so freely that it was difficult to insert eggs quickly enough to prevent them from being washed away. A total of 470 eggs inserted were sealed within the incisions with gummed labels and a thin layer of paraffin. Upon the examination of 270 eggs 2 days later, it was found that 60 eggs had hatched and that the newly hatched larvæ were alive and active within the incisions. Later examinations showed that all larvæ died without entering the pulp, even where the peel had split and exposed the latter. An examination of the 200 other eggs 9 days after they were placed within the incisions showed that 135 had hatched, but all the larvæ had died without infesting the pulp. The 275 of the 470 eggs that failed to hatch turned black. Of 65 eggs of the same lot held as a check, 57 hatched.

EXPERIMENTS IN THE LABORATORY

All experiments carried on in the laboratory necessarily were with fruits cut from the tree. The results were therefore obtained under conditions less normal than those obtained in the field. No experiments can be said to be carried on under field conditions unless the fruit is still growing, for as soon as it is cut its protecting sap begins to disappear.

One bunch of 55 fruits which had been cut for shipment for 24 hours was confined for 48 hours with about 500 fruit flies. An examination of the individual fruits after the bunch was removed from the cage showed 22 with a total of 28 punctures. These punctures were not opened, but the fruits were placed in jars. No adult fruit flies developed.

One bunch of 93 fruits, which had been cut for shipment for about 6 hours, was confined for 24 hours with about 300 fruit flies. On removal from the cage it was found that only 15 fruits were free from attempts at oviposition. In the remaining 78 fruits there were 342 punctures. Eggs were laid in only 7 of these 342 punctures. All eggs, or newly-hatched larvæ, died in 5 of the 7 punctures and only 3 adult flies succeeded in developing, in but one of the two fruits the pulp of which was found infested 5 days after the fruit was removed from the cage. The fruits on this bunch were almost too mature for shipment.

Twenty fruits from a bunch cut four days previously for shipment were confined in a jar containing about 400 fruit flies. Five fruits were

removed after 24 hours; 15 fruits after 72 hours. At the end of the 72 hours, or 7 days after the fruits were cut, they were beginning to turn color. In the peel of the 5 fruits first removed 58 punctures were made; yet only 1, 3, 2, and 1 fruit flies, respectively, were reared from 4 of the fruits. In the peel of the 15 fruits removed at the end of 72 hours there were 148 punctures, of which 28 contained eggs. Two days after the fruit was removed from the jars, the 28 punctures were found to contain 59 hatched eggs and 27 dead eggs. While punctures were found to be entirely empty in only 2 of the 15 fruits, adult fruit flies failed to mature in 7. There issued from the remaining 8 fruits an average of 2.2 flies, 8 being the largest number to emerge from a single fruit. Two fruits, found to contain 18 and 19 eggs, respectively, failed to produce adults.

Three fruits of the wild Borabora banana, which had been cut from the tree for two days and were still hard and yielding small quantities of sap when cut from the bunches, were placed with about 200 fruit flies for 24 hours. After removal from the cage, one fruit contained 56 eggs in its peel. The two other fruits were placed in rearing jars and produced 104 and 187 adult fruit flies, respectively. The pulp of the Borabora banana is very firm and does not decay as rapidly as does that of the Chinese or Bluefield banana.

Only 35 adults matured from 880 eggs taken from apples and placed in the peel of 44 bananas that had been cut for shipment for 24 hours. Of the 44 fruits only 31 produced adult fruit flies. Out of 107 newly hatched larvæ from apples, placed in the pulp of ripe bananas, but 33 succeeded in reaching the adult stage. Out of 137 newly hatched larvæ placed in the pulp of green bananas ready for shipment, but 40 completed the life cycle. Of these 137 larvæ 15, 52, 60, 26, and 10 were placed in bananas that had been cut from the tree 1, 2, 3, 4, and 9 days, respectively; the adults reared in the same order numbered 3, 12, 13, 5, and 7.

CAUSES OF IMMUNITY OF GREEN BANANAS TO FRUIT-FLY ATTACK

While it is difficult to understand why Mediterranean fruit flies have not been reared from ripe and split fruits collected on the plantations, it is not so difficult to find reasons for the immunity of fruits until they are about to turn yellow. Chemical analysis of the banana during its development, made by Mr. A. R. Thompson, of the Hawaii Agricultural Experiment Station, have shown that there exists much tannin in the peel and about the sections of which the banana fruit is composed. This tannic acid is very abundant in the green fruit, but decreases greatly in amount as the fruit becomes edible. During development, even up to the time when bananas are cut for shipment, which usually is about 12 to 16 days before they would become ripe enough to eat if kept under Hawaiian weather conditions, the peel of the fruit is so surcharged with sap laden with tannic acid that the slightest scratch of the peel produces

a flow of this staining fluid. Data on file show that practically all punctures made by female fruit flies in host fruits, the epidermis of which does not emit fluid detrimental to the pest from one or several standpoints, contain eggs, but no punctures or eggs have ever been found by the writers in the peel of bananas growing under normal field conditions and suitable for the export trade. This is true in spite of the fact that many thousand fruits have been examined.

One of the most severe tests to which any fruit can be subjected to determine whether it can support the fruit fly is to confine it closely with several hundred fruit flies of both sexes. Yet even under this extreme and unnatural condition only 1 egg was laid in 1,449 bananas exposed while still attached to the tree, and that was killed, presumably by the tannic acid in the peel. While 22 eggs were deposited in 1,145 more mature fruits, also attached to the tree, some of which were too mature for export trade, these eggs, or the larvæ hatching from them, died within the peel. When one realizes that many thousand eggs have been secured by the writers under like conditions in preferred hosts, it is clear that adult fruit flies find it extremely difficult to oviposit in fruits on the tree even under forced conditions, both when the fruit is sufficiently mature for shipment and for a period of at least nine days thereafter. At the end of this period it is considered too mature to stand transportation to the mainland. And inasmuch as shippers are paid by the bunch for their fruit, the banana markers in Hawaii are likely to mark bananas for cutting that are slightly greener than necessary in order to safeguard against unforeseen delays in shipment and crowded conditions on board the steamer which hasten the ripening process.

The difficulty experienced by the female Mediterranean fruit flies in ovipositing in green though mature fruit still attached to the tree is undoubtedly a mechanical one. She no sooner ruptures the epidermis in her attempt to form a cavity within which to deposit her eggs than she is literally forced away from her position by the exuding sap. It is possible that repeated attempts at oviposition, which are known to occur in other host fruits under natural conditions, may account for the 7 instances out of the 494 under forced or abnormal conditions when females were successful in depositing eggs. That the immunity enjoyed by Chinese and Bluefield bananas up to the time they are ready for shipment and for a period of at least nine days thereafter is due to the copious supply of sap is still further emphasized by the ease with which they become infested under similar forced conditions, or outdoor conditions, when the fruit has been cut for a short time. Fruit cut from the tree or from the bunch bleeds at the point where severed. The pressure of sap within is at once reduced and the amount of sap that exudes from cuts in the peel decreases until but little exudes after the fruit has been cut for several days. The data giving the results of close confinement of flies with bananas after they have been cut for shipment show that while the females have diffi-

culty in ovipositing as abundantly as they would in preferred hosts, such as the apple and peach, yet they find little difficulty in depositing a sufficient number of eggs to infest slightly a few of the fruits.

Inasmuch as not a single egg or newly hatched larva, as recorded in the data, was able to live in the tannin-laden peel of green though mature bananas still attached to the tree, while adults were frequently able to reach maturity in fruits severed from the tree, from which much of the sap had been drained or altered by chemical changes that proceed with the ripening process, it is evident that the sap is the chief cause of the immunity of bananas to the attack of *Ceratitidis capitata*.

There is no danger of infestation during the interval between the time bananas are cut in the field and the time they are wrapped for shipment in the packing sheds.

It has been noted that oviposition has taken place under forced conditions within from 6 to 24 hours after the fruits have been cut from the tree, but that eggs deposited under such conditions have either died or the larvæ hatching from these have died without reaching the pulp. This leads to the question whether there is not danger of bananas becoming infested between the time when they are cut and the time when they are wrapped. The writers have never seen adult flies resting on bananas cut and stacked in the packing sheds, although they have personally seen many thousands of bunches ready for inspection during a 3-year period. Trade requirements demand that fruits be cut as late before the date of steamer sailing as possible. It therefore happens that bunches of bananas are inspected and wrapped within from 2 to 24 hours after they are cut, and this prompt wrapping removes all danger of infestation (Pl. LX, fig. 1, 2). From the fact that no infestation of growing bananas in condition for shipment has been known to occur in Hawaii, and that such infestations in cut fruits also suitable for shipment that are recorded have been obtained under forced conditions, whereas they have been found lacking under normal conditions, the writers believe that there is no possibility of infestation taking place between the time of cutting and that of wrapping.

OBSERVATIONS AND EXPERIMENTS OF OTHER ENTOMOLOGISTS

Kirk, of New Zealand, lists (4) the banana among fruits from Australia, condemned in New Zealand, in which the maggots of the fruit fly¹ had

¹ From the arrangement of the text of Kirk's bulletin (4), the Mediterranean fruit fly (*Ceratitidis capitata*) is definitely listed as a banana pest. The bulletin is, however, a compilation taken for the most part verbatim from various articles on fruit flies appearing in the Reports of the Agricultural Department of New Zealand, or from circulars issued by the department. A person unfamiliar with the Australian situation is at a loss to know to which of several fruit-fly pests reference is made in the reports of fruits found infested by maggots at the ports of entry. Thus, in the Thirteenth Volume of the Agricultural Reports, 1905, where the list including the banana among those fruits found infested was originally published, no reference is made to either the Queensland or the Mediterranean fruit fly; it is merely stated that the fruits listed were burned because found infested with the "dreaded maggot." In the report for 1906 it is definitely stated that only the Queensland fruit fly (*Dacus tryoni*) was reared that year from a list of fruits including the banana. The biologist of Western Australia in his report (1) for the year 1898 stated that the Queensland fruit fly had been brought to Western Australia in bananas.

been found. French, of Victoria, Australia, states (3) that adults of this pest were reared from bananas (*Musa* sp.) exported from Queensland, Australia, and that on many occasions he has proved eggs to have been deposited in green bananas before shipment from Queensland to Melbourne. Both Kirk and French are aware that the Queensland fruit fly (*Dacus tryoni*) is a pest of bananas grown in Queensland and that confusion between the two fruit flies might occur if observations were made by untrained inspectors.

The only actual data, aside from those presented in this paper, giving the results of experimental work to determine the status of the banana as a host fruit of the Mediterranean fruit fly have been presented by Severin and Hartung (5, 6). This work was done in Honolulu and the results are of such value that they should be consulted by those interested. Their experiments, however, were carried on with fruits detached from the tree, and when green fruits were used no statement regarding the degree of greenness was made. In view of the fact that they reared specimens of the fruit fly from only two fruits out of "hundreds of bunches of bananas" examined on trees cut down in Honolulu during a campaign against mosquitoes, the writers seriously question the statement made by Severin in a later publication (7) that the "fruit fly was also bred from a half-ripe banana under field conditions." The fact that Severin reared numerous specimens of the decay flies, *Acritochaeta pulvinata*, *Euxesta annonae* Fab., and *Notogramma stigma* Fab., besides a number of species of Drosophilidae, is ample evidence that the trees from which the two fruits were taken had been cut sufficiently long for decay to have started in many fruits, had he not stated that one of the two fruits from which he reared adult flies was in a bruised and decaying condition and that its pulp had already turned yellow beneath the decayed area. It is general knowledge in Honolulu that such quantities of bearing banana trees were cut down during the campaign mentioned that the city garbage department was completely demoralized and that the trees with their fruit attached were stacked along the streets in certain parts of the city for over a week, thus giving fruit flies an opportunity to oviposit under, not growing or field, but abnormal conditions.

CONCLUSIONS

Since the Mediterranean fruit fly (*Ceratitis capitata* Wied.) has not been found infesting the Chinese banana (*Musa cavendishii*) or the Bluefield banana (*Musa* sp.) during the three years that the Federal Government has had charge of the inspection of export bananas in the Hawaiian Islands, it is evident that some reason exists for this practical immunity. This is the more apparent since adult flies of both sexes have been found present in all parts of banana plantations, and surrounding fruits known to be hosts have been heavily infested.

This immunity is shown to be due to the fact that neither the egg nor the newly hatched larva of the fruit fly can survive in the tannin-laden peel of green though mature fruit. In fact, the copious and sudden flow of sap from egg punctures made by fruit flies in unripe bananas renders the successful deposition of eggs in such fruits difficult and rare.

The fact that not 1 of 1,044 fruits of the Chinese banana ripening singly and prematurely among bunches growing in the field, and upon which, as in the case of other host fruits, one might expect gravid females to concentrate their attention for the purpose of oviposition, has been found to be infested leads to the conclusion that even ripe bananas are not desired as host fruits by adult fruit flies under Hawaiian conditions. On the other hand, the rearing of flies from the ripe and yellow fruits of the thin-skinned Popoulu variety, as well as from ripe fruits of other varieties under forced and unnatural conditions, leads to the equally acknowledged fact that ripe bananas in the field may serve as hosts and should therefore be properly guarded against in all quarantine work.

From the facts stated the writers believe that bunches of any variety of banana now growing in the Hawaiian Islands, when properly inspected for the removal of prematurely ripe, cracked, or partially decayed fruits, offer no danger as carriers of the Mediterranean fruit fly, provided they are wrapped and shipped in accordance with the demands of the trade and the Federal regulations.

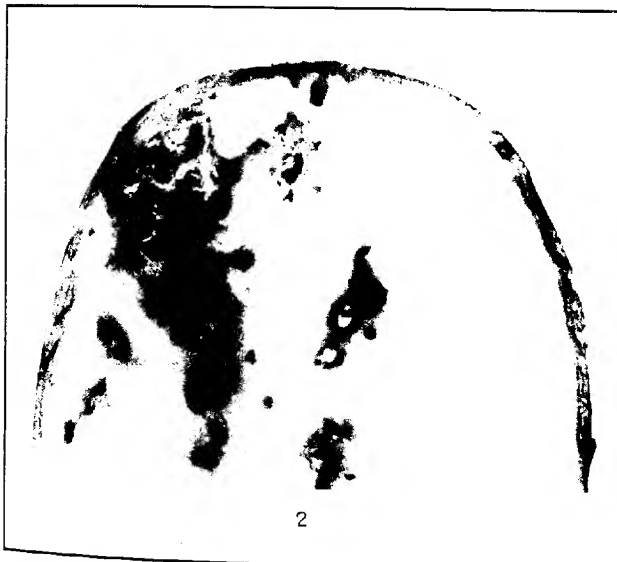
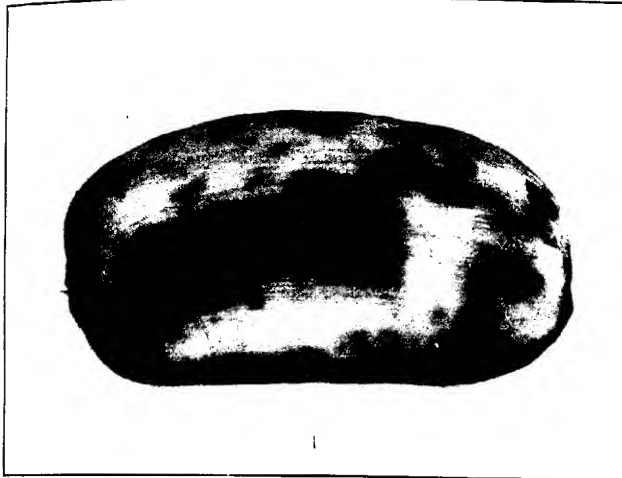
LITERATURE CITED

- (1) HELMS, R.
1899. Report of the biologist. *In* Rpt. Dept. Agr. [West. Aust.], [July]/Dec. 1898, p. 17-20.
- (2) HIGGINS, J. E.
1904. The banana in Hawaii. *Hawaii Agr. Exp. Sta. Bul.* 7, 53 p., 9 pl., 9 fig.
- (3) FRENCH, C.
1907. Fruit flies. *In* Jour. Dept. Agr. Victoria, v. 5, no. 5, p. 301-312, 1 pl.
- (4) KIRK, T. W.
1909. Fruit flies. *New Zealand Dept. Agr. Div. Biol. Bul.* 22, 18 p., 2 fig.
- (5) SEVERIN, H. H. P., and HARTUNG, W. J.
1912. Will the Mediterranean fruit fly (*Ceratitis capitata* Wied.) breed in bananas under artificial and natural conditions? *In* Mo. Bul. State Com. Hort. [Cal.], v. 1, no. 9, p. 566-569.
- (6) ———
1912. Will the Mediterranean fruit fly (*Ceratitis capitata* Wied.) breed in bananas under artificial and field conditions? *In* Jour. Econ. Ent., v. 5, no. 6, p. 443-451.
- (7) SEVERIN, H. H. P.
1913. Precautions taken and the danger of introducing the Mediterranean fruit fly (*Ceratitis capitata* Wied.) into the United States. *In* Jour. Econ. Ent., v. 6, no. 1, p. 68-73.
- (8) U. S. DEPARTMENT OF AGRICULTURE. FEDERAL HORTICULTURAL BOARD.
1914. Mediterranean fruit fly and melon fly. U. S. Dept. Agr. Fed. Hort. Bd. Notice of Quarantine 13, 4 p.

PLATE LIX

Fig. 1.—Popoulu variety of cooking banana found infested with the Mediterranean fruit fly. Note holes made in peel by the emerging larvæ. This fruit was fully ripe when found infested; mature fruits still green in color, present on the same bunch, were not infested.

Fig. 2.—Cross section of the Moa variety of cooking banana, showing pulp infested by larvæ of the Mediterranean fruit fly. Larvæ were found infesting the pulp of this variety only when the fruits had become mature, though not yellow in color, and when the peel had cracked sufficiently to expose the pulp, thus removing Nature's barrier to infestation.



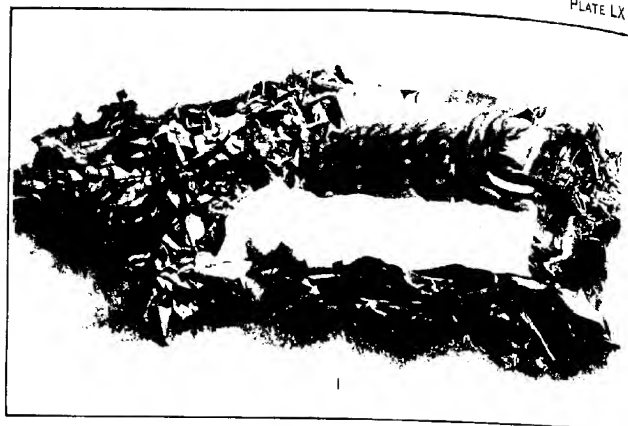


PLATE LX

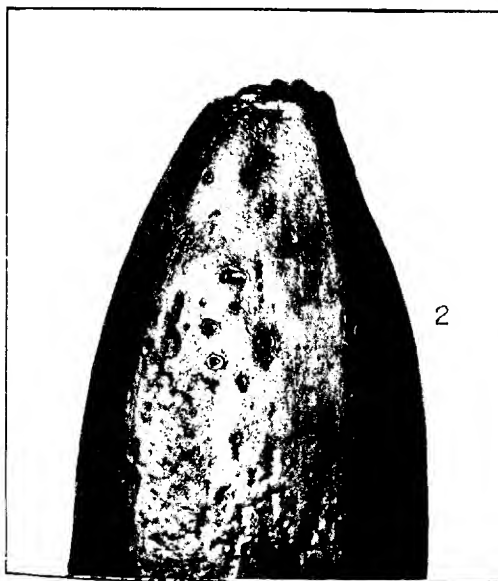
Fig. 1.—A bunch of Chinese bananas (*Musa cavendishii*). The fruit of this variety is so tender that it has to be protected during shipment by wrapping. The bunch is first wrapped in paper or cheesecloth and then in dried banana leaves, rice straw, or a mixture of the two.

Fig. 2.—A bunch of Chinese bananas wrapped in banana leaves and ready for shipment to California. Packing materials are stored for several months before use and are constantly under the supervision of inspectors to make sure that they are kept free from fruit-fly contamination.

PLATE LXI

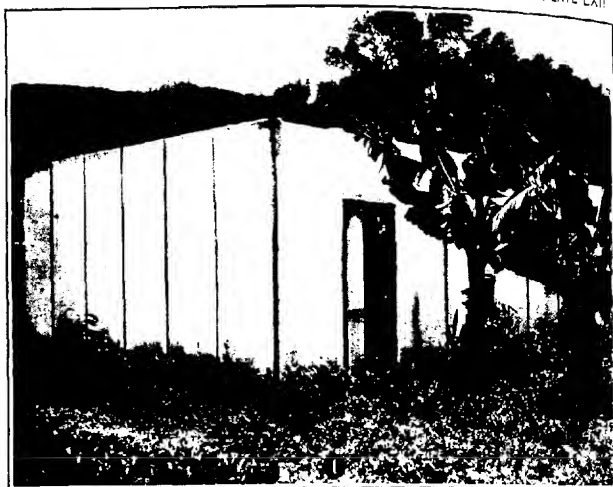
Fig. 1.—Cleaning bananas in Hawaii before shipment. Every bunch of bananas shipped from the plantations in Hawaii is carefully cleaned by the Chinese growers before being inspected for the presence of ripe, cracked, bruised, or decayed fruits.

Fig. 2.—Tip of Chinese banana (*Musa cavendishii*), showing punctures made by the female Mediterranean fruit fly in attempts to deposit eggs within the peel. Though made under forced and abnormal conditions, while the fruit was still attached to the tree, and seven to nine days after it had become sufficiently mature for shipment, the 18 punctures were empty and but 2 were deep enough to contain eggs.



Banana, Host Fruit of Mediterranean Fruit Fly

PLATE LXII



Young of *A. ...* (P. ...)

Vol. V, No. 17

PLATE LXII

Fig. 1.—Rearing cage erected over 20 Chinese banana trees and inclosing 14 bunches in various stages of development. Although adults of the Mediterranean fruit fly were introduced from time to time, none of the fruits were found infested when they became ripe.

Fig. 2.—Interior of rearing cage shown in figure 1.

EFFECT OF CONTROLLABLE VARIABLES UPON THE PENETRATION TEST FOR ASPHALTS AND ASPHALT CEMENTS

By PRÉVOST HUBBARD, *Chemical Engineer*, and F. P. PRITCHARD, *Assistant Chemist*,
Office of Public Roads and Rural Engineering

INTRODUCTION

No one test for asphalts and asphalt cements is probably better known or more generally used than the penetration test. Many instruments have been devised for determining the consistency of these materials, but none have been generally adopted that do not substantially conform to the fundamental principles of the apparatus known as the Dow penetration machine.¹ This machine and others designed to give practically equivalent results are too well known to require description in this paper. In general, however, it may be said that by their use the consistency of asphalts or asphalt cements is expressed as the depth in hundredths of a centimeter that a standard needle will penetrate them vertically without external friction while the material is maintained at a stated temperature and the needle is operated under a stated load for a stated length of time. In the Dow penetration machine external friction is practically eliminated. In other satisfactory types it is reduced to an almost negligible minimum, but when operating with those in which the needle holder slides through a guiding sleeve it is most important that both the plunger and sleeve be absolutely clean and dry, as a small amount of moisture, oil, or dirt will produce considerable friction and thus retard the penetration of the needle into the sample being tested. Certain standards of temperature, load, and time have been generally adopted, and the most widely used combination is 25° C., 100 gm., 5 seconds.

Granting that the apparatus is mechanically satisfactory and that a definite standard needle is used, the test appears to be comparatively simple. It has frequently been found, however, that different laboratories, working upon samples of the same material under supposedly identical conditions of temperature, load, and time, obtain appreciably different results. The object of this investigation has therefore been to determine what effect apparently slight differences in these conditions will produce in the results of tests and also to study the importance of other controllable variables.

¹ Dow, A. W. The testing of bitumens for paving purposes. *In* Proc. Amer. Soc. Testing Materials, 6th Ann. Meeting 1903, v. 3, p. 349-368, fig. 1-6. Discussion, p. 369-373. 1903.

The materials for this work were selected with the idea of obtaining products which showed rather wide differences in physical and chemical properties. For this purpose four types of oil asphalt were selected, which, being practically all bitumen, eliminated to a large extent variations due to sampling, which might have occurred in the case of native asphalts or fluxed native asphalts carrying appreciable quantities of non-bituminous material. The types represented in the following tables are produced from (1) steam-refined California petroleum, (2) steam-refined Mexican petroleum, (3) refined blended petroleum, and (4) blown petroleum. Three grades of each type were selected, having, at 25° C., under a load of 100 gm. applied for 5 seconds, penetrations of approximately 50, 100, and 150. This made 12 samples in all, and it is believed that the results obtained by their use can consistently be interpreted to cover practically all types of asphalts and asphalt cements. The more important physical and chemical characteristics of these products are shown in Table I.

TABLE I.—Characteristics of asphalt cements

Test.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8997	8998	8999
Specific gravity, 25°/25° C.	1.039	1.036	1.036	1.048	1.046	1.036	1.026	1.025	1.031	0.993	0.988	0.987
Melting point (cube method).....	53° C.	46° C.	42° C.	62° C.	52° C.	46° C.	62° C.	58° C.	44° C.	114° C.	82° C.	68° C.
Penetration, 25° C., 100 gm., 5 sec.	47	93	133	50	90	150	62	92	157	44	91	150
Penetration, 0° C., 200 gm., 1 min.	3	12	18	13	26	40	22	36	39	27	47	49
Penetration, 46° C., 50 gm., 5 sec.	Soft	Soft	Soft	227	Soft	Soft	270	310	Soft	70	176	295
Loss, 103° C., 20 gm., 5 hrs.	.77	.90	1.28	.09	.16	.46	.38	.87	.99	.14	.20	.21
Penetration residue, 25° C., 100 gm., 5 sec.	26	45	61	37	55	87	45	58	92	38	86	118
Bitumen-soluble (CS ₂)	99.82	99.46	99.74	99.64	99.92	99.95	99.66	99.92	99.82	99.54	99.61	99.88
Organic-insoluble	.06	.36	.20	.10	.06	.05	.21	.07	.13	.27	.21	.09
Inorganic-insoluble	.12	.18	.06	.06	.02	.00	.13	.01	.05	.19	.18	.12
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Bitumen insoluble, 80° B. m.p.	21.4	19.9	18.0	29.8	29.8	25.7	29.1	28.9	25.7	25.5	24.8	22.7
Fixed carbon	13.4	13.3	11.6	18.1	17.6	15.3	14.0	14.2	14.5	15.5	12.4	11.3

The first consideration which naturally presents itself is the method of preparing the sample for the test. It is apparent that in order to duplicate results upon different samples of the same material the samples shall be taken so as to represent the entire body of material sampled.

It is assumed that in all instances laboratories take representative samples. The handling of the sample, once it is taken, however, is subject to a number of conditions which are not ordinarily strictly specified. In the first place, the sample must be melted by the application of heat and, to prevent any change during the melting process, it should be heated at as low a practicable working temperature as consistent with the time required to melt it. That is, all asphalts and asphalt cements tend to harden upon being heated, due either to loss by volatilization or to so-called oxidation or reaction with atmospheric air. This tendency is increased as both the temperature and time of melting are increased. The method followed in preparing all of the samples for this investigation was as follows:

About 6 ounces of each of the 12 materials were placed in pint tin cups. The 12 cups were then placed upon a $\frac{1}{4}$ -inch asbestos board resting directly upon a gas hot plate. The samples were stirred occasionally to expedite melting, and removed from the hot plate as soon as completely fluid. At no time were the samples heated sufficiently to produce fuming. Upon removal from the hot plate the samples were poured into 3-ounce cylindrical tin dishes, measuring 5.5 cm. in diameter, with vertical sides approximately 3.5 cm. in height. While still fluid, all air bubbles which rose to the surface were removed by means of a tiny gas flame, which was rapidly passed over the surface and which merely caused the bubbles to break without in any way injuring the sample.

As the effect of the size of the container upon the results of tests had been investigated by Reeve,¹ it was felt that by the use of the dish above stated no danger of influencing results from this cause need be feared. In this connection it is of interest to note that Reeve's work demonstrated that a dish of 5 cm. or more in diameter could not influence the results of tests, although appreciable variations in results were in some cases caused by dishes smaller than 2.5 cm. in diameter.

EFFECT OF VARIATIONS IN METHOD OF PREPARING MELTED SAMPLES FOR TESTING

Undoubtedly the most common method of preparing a melted sample for the penetration test is to allow it to cool in air at room temperature for approximately an hour, then to immerse it for an hour in water maintained at the temperature at which the test is to be made. The sample is then tested under water at this temperature. In certain cases, cooling the sample in ice water or crushed ice prior to immersing it in the constant-temperature bath has been resorted to, and the penetrations so obtained have frequently been somewhat lower than those obtained by the method first described. As great a difference as 15 points in one asphalt cement

¹ Reeve, C. S. Effect of diameter of bitumen holder on the penetration test. *In Proc. Internat. Assoc. Testing Materials* (6th Cong. New York 1912), v. 2, no. 11, Paper 25, 4 p. 1912.

of about 150 penetration has been noted by the authors in this connection. The theory has been advanced that the ice-water cooling produces a set in the material which is not attained by the sample if it is allowed to air-cool until it has stood for a number of days. It has been further argued that the penetration at this set represents more accurately the true consistency of the material than does the penetration determined by the method first described. In order to study this matter thoroughly, different samples of each of the 12 materials were cooled and prepared for testing in a variety of ways, careful attention being paid to the time during which the sample was subjected to a given condition. These conditions are shown in Table II.

For each test under a given set of conditions samples of materials were melted and poured at the same time. In methods 1 to 6 and 15 to 23, inclusive, the melted samples were poured into the test dishes and, after standing in air for the periods indicated, were immersed in a water bath carefully maintained at 25° C. for the time selected, prior to determining their penetration. At the expiration of this time they were tested in the water bath. In methods 7 to 10, inclusive, the melted samples were poured into test dishes which had been previously packed in ice. Here they were allowed to remain until transferred to the 25° water bath. In methods 11 to 14, inclusive, the melted samples were first poured into the test dishes and allowed to cool in air as indicated, after which they were placed in an ice-water bath for definite periods of time and then immediately transferred to the 25° water bath. In methods 24 and 25, the melted samples were poured into test dishes packed in crushed ice and kept there for 1 hour. They were then removed and allowed to remain in air for 28 days, after which they were placed in the 25° water bath just prior to testing as indicated.

TABLE II.—Individual penetration tests on asphalt cements, 100 gms., 5 seconds, 25° C.

Meth- od No.	Conditions before test.			California.			Mexican.			Blended.			Blown.		
	In air.	In ice.	In 25° C. Bath.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
1.....	30 min.	30 min.	57	54	50	13	114	100	177	158	143	60	57	53
2.....	30 min.	1 hr.	49	47	47	85	93	91	128	134	132	51	51	51
3.....	30 min.	1½ hrs.	47	47	47	85	93	91	128	134	132	51	51	51
4.....	1 hr.	30 min.	51	49	48	100	96	94	145	141	135	53	51	50
5.....	1 hr.	1 hr.	47	46	46	94	94	91	130	129	131	51	50	50
6.....	1 hr.	1½ hrs.	48	46	46	95	95	93	134	134	134	51	51	51
7.....	30 min.	1 hr.	48	47	45	94	93	90	129	127	127	50	50	50
8.....	1 hr.	1½ hrs.	47	46	46	93	93	93	131	131	131	49	49	49
9.....	1 hr.	1 hr.	46	46	46	93	93	93	131	131	131	49	49	49
10.....	30 min.	1 hr.	49	46	45	94	94	94	131	128	128	50	49	48
11.....	30 min.	1½ hrs.	48	47	46	93	93	93	131	132	132	49	48	48
12.....	1 hr.	1 hr.	47	45	46	94	94	94	133	133	133	48	47	47
13.....	1 hr.	1½ hrs.	47	48	47	94	94	94	133	133	133	48	47	47
14.....	24 hrs.	30 min.	44	43	43	94	94	94	133	133	133	46	47	48
15.....	24 hrs.	1 hr.	45	45	44	94	94	94	133	133	133	46	47	48
16.....	24 hrs.	1½ hrs.	45	45	45	94	94	94	133	133	133	46	47	48
17.....	3 days.	1 hr.	45	44	44	94	94	94	133	133	133	46	47	48
18.....	3 days.	1½ hrs.	44	44	44	94	94	94	133	133	133	46	47	48
19.....	7 days.	1 hr.	43	41	40	86	82	81	126	127	127	43	44	44
20.....	7 days.	1½ hrs.	43	43	43	85	85	85	125	125	125	43	43	43
21.....	1 hr.	1 hr.	38	38	39	86	86	86	120	118	118	40	40	40
22.....	1 hr.	1½ hrs.	38	38	38	79	79	79	118	119	119	39	39	39
23.....	20 days.	1 hr.	36	37	37	86	86	86	118	118	118	39	39	39
24.....	20 days.	1 hr.	37	37	37	79	79	79	118	118	118	39	39	39
25.....	25 days.	1½ hrs.	37	37	37	79	79	79	118	118	118	39	39	39

Table II gives the results of three determinations for each sample under each of the conditions tried. These penetrations were all taken with the same needle at different points on the surface of the sample. Reading from left to right, the first test was made at the center, the third 1 cm. from the edge of the dish, and the second halfway between the positions of the first and third tests. For the dish measuring 5.5 cm. in diameter, the first penetration was therefore taken 2.7 cm., the second about 1.9 cm., and the third about 1 cm. from the edge of the dish.

It will be noted that the time elapsing between pouring the sample into the dish and determining its penetration varied from a total of 1 hour to over 28 days; that the immersion in the water bath directly preceding the test varied from 30 minutes to 1½ hours. Upon reviewing the results given in this table, it appears evident that, in general, for any given set of conditions preceding the immersion in the water bath, a 30-minute immersion in water gave less consistent check results than a corresponding 1-hour or 1½-hour immersion. Less difference is indicated between the 1-hour and 1½-hour immersions in water, but the balance of evidence appears to favor the latter period of time in so far as uniformity is concerned, even when negligible personal errors are taken into account. Thus, out of the 11 series of comparative tests of 1 hour and 1½ hours for all 12 materials, it will be found that in 61 cases the 1½-hour immersion gave the most consistent results; in 21 cases the most consistent results were obtained with the 1-hour immersion; and in 50 cases there is no preference so far as consistency in results was concerned.

If the average of the three tests for any sample is taken for the 1-hour air cooling and 1-hour immersion in the bath, as compared with the 30-minute air cooling and 1-½-hour immersion in the bath, it will be found that they practically coincide. The fact, however, that in the latter case there is less difference between the individual results indicates that the 1½-hour immersion should have preference.

Eliminating the 30-minute immersion in the bath before making the test, and considering only the 1-hour and 1½-hour immersions in connection with short periods of prior cooling in air, Table III will be found to illustrate the differences above described. Here, comparing methods 5 and 3, it will be seen that in seven cases the most consistent results were obtained by the 1½-hour immersion; in two cases the 1-hour immersion produced the most consistent results; and in three cases there is no preference with regard to consistency in results. So far as rapidity in making the test is concerned, therefore, if a short-period air immersion is to be adopted, it would seem that 30 minutes in the air and 1½ hours in the bath prior to testing would be the most satisfactory minimum limits to adopt.

TABLE III.—Comparison of penetration tests for short periods of air cooling and immersion in 25° C. bath, 100 gm., 5 seconds

Method No.	Conditions before test.		California.			Mexican.			Blended.			Blown.		
	In air.	In 25° C. bath.	8063	8062	8061	8048	8049	8020	8094	8095	8096	8097	8098	
1	49	47	91	91	138	134	132	52	51	91	137	128	51	
2	47	46	94	93	131	130	129	51	50	93	90	150	149	
3	47	46	94	93	131	130	129	51	50	93	90	150	149	
4	47	47	93	93	133	133	133	50	51	90	90	150	151	
5	47	47	93	93	133	133	133	50	51	90	90	150	151	
6	47	47	93	93	133	133	133	50	51	90	90	150	151	
7	47	47	93	93	133	133	133	50	51	90	90	150	151	
8	47	47	93	93	133	133	133	50	51	90	90	150	151	
9	47	47	93	93	133	133	133	50	51	90	90	150	151	
10	47	47	93	93	133	133	133	50	51	90	90	150	151	
11	47	47	93	93	133	133	133	50	51	90	90	150	151	
12	47	47	93	93	133	133	133	50	51	90	90	150	151	
13	47	47	93	93	133	133	133	50	51	90	90	150	151	
14	47	47	93	93	133	133	133	50	51	90	90	150	151	
15	47	47	93	93	133	133	133	50	51	90	90	150	151	
16	47	47	93	93	133	133	133	50	51	90	90	150	151	
17	47	47	93	93	133	133	133	50	51	90	90	150	151	
18	47	47	93	93	133	133	133	50	51	90	90	150	151	
19	47	47	93	93	133	133	133	50	51	90	90	150	151	
20	47	47	93	93	133	133	133	50	51	90	90	150	151	
21	47	47	93	93	133	133	133	50	51	90	90	150	151	
22	47	47	93	93	133	133	133	50	51	90	90	150	151	
23	47	47	93	93	133	133	133	50	51	90	90	150	151	
24	47	47	93	93	133	133	133	50	51	90	90	150	151	
25	47	47	93	93	133	133	133	50	51	90	90	150	151	
26	47	47	93	93	133	133	133	50	51	90	90	150	151	
27	47	47	93	93	133	133	133	50	51	90	90	150	151	
28	47	47	93	93	133	133	133	50	51	90	90	150	151	
29	47	47	93	93	133	133	133	50	51	90	90	150	151	
30	47	47	93	93	133	133	133	50	51	90	90	150	151	
31	47	47	93	93	133	133	133	50	51	90	90	150	151	
32	47	47	93	93	133	133	133	50	51	90	90	150	151	
33	47	47	93	93	133	133	133	50	51	90	90	150	151	
34	47	47	93	93	133	133	133	50	51	90	90	150	151	
35	47	47	93	93	133	133	133	50	51	90	90	150	151	
36	47	47	93	93	133	133	133	50	51	90	90	150	151	
37	47	47	93	93	133	133	133	50	51	90	90	150	151	
38	47	47	93	93	133	133	133	50	51	90	90	150	151	
39	47	47	93	93	133	133	133	50	51	90	90	150	151	
40	47	47	93	93	133	133	133	50	51	90	90	150	151	
41	47	47	93	93	133	133	133	50	51	90	90	150	151	
42	47	47	93	93	133	133	133	50	51	90	90	150	151	
43	47	47	93	93	133	133	133	50	51	90	90	150	151	
44	47	47	93	93	133	133	133	50	51	90	90	150	151	
45	47	47	93	93	133	133	133	50	51	90	90	150	151	
46	47	47	93	93	133	133	133	50	51	90	90	150	151	
47	47	47	93	93	133	133	133	50	51	90	90	150	151	
48	47	47	93	93	133	133	133	50	51	90	90	150	151	
49	47	47	93	93	133	133	133	50	51	90	90	150	151	
50	47	47	93	93	133	133	133	50	51	90	90	150	151	
51	47	47	93	93	133	133	133	50	51	90	90	150	151	
52	47	47	93	93	133	133	133	50	51	90	90	150	151	
53	47	47	93	93	133	133	133	50	51	90	90	150	151	
54	47	47	93	93	133	133	133	50	51	90	90	150	151	
55	47	47	93	93	133	133	133	50	51	90	90	150	151	
56	47	47	93	93	133	133	133	50	51	90	90	150	151	
57	47	47	93	93	133	133	133	50	51	90	90	150	151	
58	47	47	93	93	133	133	133	50	51	90	90	150	151	
59	47	47	93	93	133	133	133	50	51	90	90	150	151	
60	47	47	93	93	133	133	133	50	51	90	90	150	151	
61	47	47	93	93	133	133	133	50	51	90	90	150	151	
62	47	47	93	93	133	133	133	50	51	90	90	150	151	
63	47	47	93	93	133	133	133	50	51	90	90	150	151	
64	47	47	93	93	133	133	133	50	51	90	90	150	151	
65	47	47	93	93	133	133	133	50	51	90	90	150	151	
66	47	47	93	93	133	133	133	50	51	90	90	150	151	
67	47	47	93	93	133	133	133	50	51	90	90	150	151	
68	47	47	93	93	133	133	133	50	51	90	90	150	151	
69	47	47	93	93	133	133	133	50	51	90	90	150	151	
70	47	47	93	93	133	133	133	50	51	90	90	150	151	
71	47	47	93	93	133	133	133	50	51	90	90	150	151	
72	47	47	93	93	133	133	133	50	51	90	90	150	151	
73	47	47	93	93	133	133	133	50	51	90	90	150	151	
74	47	47	93	93	133	133	133	50	51	90	90	150	151	
75	47	47	93	93	133	133	133	50	51	90	90	150	151	
76	47	47	93	93	133	133	133	50	51	90	90	150	151	
77	47	47	93	93	133	133	133	50	51	90	90	150	151	
78	47	47	93	93	133	133	133	50	51	90	90	150	151	
79	47	47	93	93	133	133	133	50	51	90	90	150	151	
80	47	47	93	93	133	133	133	50	51	90	90	150	151	
81	47	47	93	93	133	133	133	50	51	90	90	150	151	
82	47	47	93	93	133	133	133	50	51	90	90	150	151	
83	47	47	93	93	133	133	133	50	51	90	90	150	151	
84	47	47	93	93	133	133	133	50	51	90	90	150	151	
85	47	47	93	93	133	133	133	50	51	90	90	150	151	
86	47	47	93	93	133	133	133	50	51	90	90	150	151	
87	47	47	93	93	133	133	133	50	51	90	90	150	151	
88	47	47	93	93	133	133	133	50	51	90	90	150	151	
89	47	47	93	93	133	133	133	50	51	90	90	150	151	
90	47	47	93	93	133	133	133	50	51	90	90	150	151	
91	47	47	93	93	133	133	133	50	51	90	90	150	151	
92	47	47	93	93	133	133	133	50	51	90	90	150	151	
93	47	47	93	93	133	133	133	50	51	90	90	150	151	
94	47	47	93	93	133	133	133	50	51	90	90	150	151	
95	47	47	93	93	133	133	133	50	51	90	90	150	151	
96	47	47	93	93	133	133	133	50	51	90	90	150	151	
97	47	47	93	93	133	133	133	50	51	90	90	150	151	
98	47	47	93	93	133	133	133	50	51	90	90	150	151	
99	47	47	93	93	133	133	133	50	51	90	90	150	151	
100	47	47	93	93	133	133	133	50	51	90	90	150	151	

This being so, the average of results given in Table II can best be considered by means of Table IV, in which are given the average penetrations obtained on all of the samples under various conditions of cooling prior to $1\frac{1}{2}$ hours' immersion in water. A study of this table shows in every case a gradual hardening or lowering of penetration as the time in air is increased. This lowering in penetration is not very pronounced in a period of 24 hours, but it increases quite appreciably in longer periods. Allowing for slight experimental errors, no difference is found to exist between the 30-minute and 1-hour exposure in air. The most marked difference is, of course, apparent between the results of 28 days in air as compared with 30 minutes in air, and the greatest difference in actual points of penetration will in every case, for a given type of material, be found for the softest grade of that type, or, in other words, for that grade which originally showed the highest penetration. It is apparent that no permanent set occurs up to a period of 28 days, but that a gradual hardening takes place. This being so, it is of interest to compare the foregoing with the results obtained by immersion in ice water prior to immersion in the water bath for $1\frac{1}{2}$ hours at 25°C . It will be seen, in general, that but little difference in results is obtained between the samples cooled in ice water and those cooled in air, although under certain conditions for the short periods a slightly lower penetration has been secured by this means. It is safe to say, however, that the immersion of the sample in ice water does not produce a set which is comparable to any definite set produced by prolonged standing in air. This is evident from the last series of results, in which the samples which had been immersed in ice water for an hour were allowed to stand 28 days before immersing them in the water bath, the results in each case being appreciably lower than those obtained by immersing them for 1 hour in ice water and then $1\frac{1}{2}$ hours in the bath just prior to test. There does not therefore, appear to be any good reason for cooling the sample in ice water at any time, except, perhaps, in plant-control work, where it is desired to expedite the test somewhat, and an allowance can be made for variations from the ordinary method caused by the ice-water immersion.

TABLE IV.—Comparison of average penetrations at 25°C . after $1\frac{1}{2}$ hours' immersion in bath, 100 gm., 5 seconds

Conditions before test.			California.			Mexican.			Blended.			Blown.		
In air.	In ice.	In air.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
.....	30 min.	47	93	133	50	90	150	62	92	157	44	91	136
.....	1 hr.	46	95	134	50	90	147	61	93	158	42	91	136
.....	24 hrs.	45	93	131	47	86	142	56	88	150	41	89	133
.....	3 days.	44	90	130	45	83	139	54	85	144	40	88	128
.....	7 days.	43	85	125	45	78	131	53	82	142	42	85	128
.....	28 days.	38	79	119	39	70	124	48	73	131	38	75	112
28 days (re-melted)	30 min.	46	95	133	49	91	148	61	89	156	42	93	136
.....	30 min.	47	93	131	49	90	151	61	94	157	43	91	135
.....	1 hr.	47	94	133	49	87	146	60	92	152	42	91	136
30 min.	30 min.	46	93	132	49	88	146	60	93	153	43	91	135
30 min.	1 hr.	47	92	133	48	87	142	59	92	150	43	92	134
.....	1 hr.	28 days.	37	79	119	38	68	124	48	74	131	37	75	111

Although all of the samples examined hardened very materially upon setting for 28 days, it is of interest to note that when these samples were remelted, allowed to cool in air for 30 minutes, immersed in the water bath at 25° C. for 1½ hours, and again tested, the penetrations, to all intents and purposes, were the same as those originally obtained by the 30-minute air cooling and 1½-hour immersion in the bath. This fact does not, however, indicate that the materials do not permanently harden with age, as Hubbard and Reeve¹ have shown that all types of bitumen permanently harden upon prolonged exposure.

As a result of the foregoing observations, the 30-minute air cooling and 1½-hour immersion in the bath prior to the test was adopted as the method of preparing samples prior to studying the effect of the variables, temperature, load, and time.

EFFECT OF VARIATIONS IN TEMPERATURE

The penetration of an asphalt cement is frequently determined and sometimes specified at three temperatures. The temperature most commonly employed and at which the consistency of the material is rated is 25° C. This is known as normal temperature, and the customary load and time factors used are 100 gm. and 5 seconds.

The penetration test is next frequently made at 0° C. with a load of 200 gm. applied for 1 minute. In some cases the test may be made with a load of 100 or 200 gm. applied for 5 seconds. For this test the sample is usually packed in finely crushed ice, which completely covers it, and the needle is brought in contact with its upper surface through a hole in the ice worked out with the finger. The needle itself, as well as the exposed surface, may, therefore, at the time of test be at a somewhat higher temperature than 0°. For this reason 4° C. has been selected by some for a low-temperature test, as it is a temperature which may be accurately maintained in the water bath.

Another temperature at which the penetration test is made is 46° C. Where possible, a load of 50 gm. is applied for 5 seconds, but in the case of materials which are very soft at this temperature the 50-gm. load is applied for 1 second.

In order to study the effect of variations in temperature upon the penetration test, a number of samples of each of the 12 asphalt cements were prepared, and after cooling in air for 30 minutes were placed for 1½ hours in the bath maintained at the test temperature. The results of these tests are given in Table V.

¹ Hubbard, Prévost, and Reeve, C. S. The effect of exposure on bitumens. *Trans. Jour. Indus. and Engin. Chem.*, v. 5, no. 1, p. 15-18, fig. 1-7. 1913.

TABLE V.—Effect of variations in temperature on penetration of asphalt cements

Temperature.	Conditions at test.			California.			Mexican.			Blended.			Blown.		
	Load.	Time.	Bath.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8996	8957	8958
° C.	Gm.	Seconds.													
20.....	100	5	Water.	24	47	69	29	55	87	38	61	94	32	63	90
23.....	100	5	...do...	37	71	106	40	73	115	50	77	126	38	81	120
24.....	100	5	...do...	40	80	115	45	81	126	53	80	136	40	84	121
24.6.....	100	5	...do...	46	86	121	47	86	136	56	85	145	44	89	129
25.....	100	5	...do...	46	92	132	49	91	142	60	90	156	44	91	134
26.....	100	5	...do...	53	100	149	54	99	153	65	97	169	47	98	144
27.....	100	5	...do...	60	120	172	58	106	174	68	105	187	50	107	153
0.....	100	5	Ice....	1	10	13	10	16	23	8	11	13	11	17	20
0.....	100	5	Brine.	1	3	4	4	7	10	6	10	11	8	15	20
4.....	100	5	Water.	2	5	8	7	14	17	11	17	17	14	24	31
0.....	200	5	Ice....	10	13	18	13	26	30	13	20	24	16	28	37
0.....	200	5	Brine.	2	6	8	8	13	16	12	18	20	19	29	35
4.....	200	5	Water.	7	11	15	12	17	25	16	25	27	22	39	49
0.....	200	60	Ice....	13	17	23	28	36	39	20	37	41	28	50	62
0.....	200	60	Brine.	3	12	18	13	26	40	22	36	39	27	47	59
4.....	200	60	Water.	15	25	35	18	38	59	30	48	73	36	70	89
44.....	50	1	...do...	139	239	347	99	177	268	105	182	264	45	108	175
45.....	50	1	...do...	147	263	Soft.	168	188	290	118	161	261	49	116	185
46.....	50	1	...do...	180	318	Soft.	116	204	308	121	174	266	54	124	200
47.....	50	1	...do...	189	Soft.	Soft.	126	224	Soft.	130	190	Soft.	56	129	211
44.....	50	5	...do...	294	Soft.	Soft.	195	330	Soft.	190	277	Soft.	64	155	264
45.....	50	5	...do...	318	Soft.	Soft.	204	Soft.	Soft.	209	286	Soft.	67	165	285
46.....	50	5	...do...	Soft.	Soft.	Soft.	217	Soft.	Soft.	220	310	Soft.	70	175	295
47.....	50	5	...do...	Soft.	Soft.	Soft.	245	Soft.	Soft.	244	Soft.	Soft.	73	186	Soft.

* In this and succeeding tables it will be noted that at 25° C. under a load of 100 gm. applied for 5 seconds, sample 8950 shows a materially lower penetration than in Tables II, III, and IV. No satisfactory explanation has as yet been found for this variation, as the maximum difference of eight points is too large to be attributed to experimental error. Numerous checks have been made upon the later results, which were obtained about three months after the first determinations. It is possible that the material had undergone some change during that period.

Considering first those tests made with a 100-gm. load applied for 5 seconds at temperatures ranging from 20° to 27° C., it will be seen that a difference of 1 degree makes a very decided difference in the recorded penetrations. In fact, the difference in penetration for all but the blown products and the harder grades of the other types is quite marked between 24.6° and 25°. Allowing for experimental errors, this difference of 0.4° is, in the case of sample 8963, responsible for a difference of 10 points' penetration. In general, the softer the material the greater the difference for any type. As specifications for the penetration at 25° of asphalt cements are frequently limited to a variation of 10 points, it is at once apparent that the temperature of the bath should be carefully maintained at the exact temperature required, and that accurately calibrated thermometers, which may be read to tenths of a degree centigrade, be used for this purpose.

Considering any or all of the three sets of tests made at low temperatures it is evident that the ice method is inaccurate, inasmuch as it frequently gives a higher penetration than the corresponding result with the 4° bath. It is evident, therefore, that if the temperature of 0° is used, a brine bath which may be maintained at 0° should be employed. It is further of interest to note that marked differences in penetration for all of the types are obtained between the 0° brine test and the 4° water test. From this it is apparent that the 4° test should not, as has sometimes been done, be considered the practical equivalent of a 0° test.

With regard to penetration tests at relatively high temperatures, it is of interest to note the accentuated effect of slight variations in temperature for any given material. This is due to the fact that all of the materials are much softer at this temperature. Thus, for a 50-gm. load applied for 5 seconds a difference of 24 points' penetration for 1° C. (between 45° and 46° C.) is noted for sample 8995, while for a 100-gm. load applied for 5 seconds at 25° C. a maximum difference of 9 points' penetration for 1° (between 25° and 26° C.) is shown for the same material.

THE EFFECT OF VARIATIONS IN LOAD

The penetration of asphalt cements is most frequently determined under a load of 100 gm. Penetration machines are, however, designed so that the combined weight of needle and plunger is 50 gm. The 100-gm. load is then obtained by placing an additional 50-gm. weight upon the plunger. A 100-gm. weight may also be used with the machine, so that loads of 50, 100, 150, and 200 gm. are possible. All of these loads are occasionally used in making the penetration test. It is clear that any variation in weight due to carelessness in manufacture or to changes brought about by the replacement of the original needle will most seriously affect the smaller loads—that is, a difference of 1 gm. should produce proportionately a more marked effect where the 50-gm. load is employed than with heavier loads. A variation of 1 gm. is, of course, much larger than would ordinarily be expected to exist in different instruments, but as great a variation as this has been noted by the writers. In order to determine the effect of variation in load, penetration tests were made upon all of the 12 samples with 1-gm. variations from the 50- and 100-gm. loads, and in addition to this the penetrations at intermediate loads between 50 and 200 gm. were determined in order to ascertain just what effect would be produced in the penetration of different types of asphalt cements by changes in load when the penetrations were all made for 5 seconds at a temperature of 25° C. The results of these tests are given in Table VI.

TABLE VI.—*Effect of variations in load on penetration of asphalt cements, 25° C., 5 seconds*

Load.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8995	8997	8998
Gm.												
40.....	31	39	89	32	60	96	40	60	106	26	53	83
50.....	32	60	90	33	61	97	40	60	106	26	54	83
51.....	31	61	91	33	62	98	40	60	106	26	54	83
60.....	35	67	98	36	67	110	43	63	120	29	63	94
75.....	40	76	112	41	77	123	48	75	133	36	72	111
90.....	43	85	123	45	85	134	54	84	147	39	82	124
99.....	46	92	132	48	90	140	60	90	155	41	89	134
100.....	46	92	132	49	90	142	60	90	155	42	90	134
101.....	46	92	132	49	91	142	60	90	155	43	90	135
125.....	51	101	146	54	101	159	65	101	173	51	105	157
150.....	59	113	160	60	113	178	76	113	192	58	118	182
200.....	68	134	178	72	129	211	90	134	218	72	153	231

Upon reviewing these results it will be noted that a variation of 1 gm. in no case produces an appreciable variation in results. In fact, the greatest variation is found to be one point penetration, and, in many cases, no difference in penetration is to be observed. It is therefore obvious that errors due to the calibration of the weights are practically negligible.

In connection with the series of tests for any individual material, it is of interest to note that within certain limits the increase in penetration is almost proportional to the increase in load. In other words, practically a straight-line curve may be obtained by plotting for any material the load against the corresponding penetration and connecting these points. If this is done the projection of the line to the axis representing increments of load will not hit this axis at its intersection with the axis representing increments of penetration. In general, it appears that blown asphalts possess less surface tension and adhesiveness than steam-distilled asphalts. The penetration of a blown asphalt therefore represents more nearly the actual distance which the needle enters the sample. In the case of steam-distilled asphalts the surface of the sample is markedly depressed by the needle, and probably proportionally greater retardation of its movement is produced by material which adheres to it.

It is of interest to note that a steam-distilled asphalt having a higher penetration than a blown asphalt at 25° C. under a load of 50 gm. applied for 5 seconds may have a lower penetration than the same blown asphalt at 25° under a load of 100 gm. applied for 5 seconds. For this reason the relative penetrations of different types of asphalt do not necessarily indicate their relative hardness.

As would naturally be supposed, in general, the greatest variations in penetrations due to variations in load are obtained upon the softer materials or those showing the highest penetration at any given load. The blown products, however, show more variation than do the other types. This is probably due to the fact that the effect of surface tension and adhesion is less pronounced with the blown products than with the steam-distilled products.

It was thought unnecessary to study the effect of variations in load at other temperatures and for other periods of time, as there was no reason to suppose that the results would be different in character from those given. The changes in time and temperature would merely change the penetration of the material and should give results comparable with those obtained upon softer or harder grades of the same type.

EFFECT OF VARIATIONS IN TIME

Penetration determinations are ordinarily made for a period of 5 seconds, especially where the 100-gm. load is employed. In the case of materials which are quite hard they may be made for a period of 1 min.

ute and usually under a load of 200 gm. This is done in most 0° or 4° C. tests. If a material is normally very soft or becomes very soft at 46° a 1-second test under a load of 50 gm. may be used. The time of test may be controlled by means of a swinging pendulum, a second clock, or metronome. The last is to be preferred because it leaves the eye free to watch the test itself and at the same time incurs less chance of error.

In order to determine the effect of variations in time upon the penetration test, samples of all 12 asphalt cements were prepared and tested at 25° C. under a load of 100 gm. applied for periods ranging from 1 to 10 seconds. The results of these tests are given in Table VII, in which every value recorded represents an average of a number of determinations made directly for the intervals of time stated.

TABLE VII.—*Effect of variations in time on penetration of asphalt cements, 25° C., 100 gm.*

Time.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Seconds.												
1.....	25	50	62	26	48	77	35	53	80			
2.....	32	63	84	33	62	100	45	67	105	33	63	97
3.....	38	74	104	37	73	116	52	76	124	39	73	108
4.....	42	84	118	44	81	132	56	84	136	47	85	117
5.....	45	88	126	45	84	140	58	88	148	48	88	126
6.....	47	91	132	48	89	145	60	91	155	44	90	131
7.....	49	98	157	50	91	150	62	94	160	45	91	135
10.....	64	130	183	61	116	192	74	115	210	49	104	159

Upon reviewing these results it will be noted that for any material a greater number of points penetration is recorded for the first second than for any other one second. In general, upon the basis of a 5-second test it will be found that about 50 per cent of the penetration occurs during the first second for all but the blown type. With this type, owing probably to less surface tension and adhesion, considerably more than 50 per cent of the total 5-second penetration occurs during the first second. After the first second there is a decided tendency for the penetration to become less and less for each succeeding second. But with the softer grades of material a difference of one-half second from the 5-second test may make as much as 7 points difference in penetration. It is evident, therefore, that for accurate work in the 5-second test the time of penetration should be controlled to within less than half-second variations. From numerous tests it appears that if a metronome is used, the time of penetration may be controlled by any careful operator to within a maximum variation of one-fifth second from the selected time of test, and this is believed to be sufficient for all practical purposes.

SUMMARY AND CONCLUSIONS

For the sake of convenience, the more important conclusions regarding the method of making penetration tests, which have been reached as a result of this investigation, are summarized below.

(1) Melted samples should be cooled for not less than 2 hours prior to test, and should be tested upon the same day that they are melted, preferably after 2 or 3 hours.

(2) Samples should be maintained at the testing temperature for not less than 1 hour, and preferably for $1\frac{1}{4}$ hours prior to test.

(3) Upon standing in the air, prepared samples show a decreasing penetration, but no definite end point or set is produced up to 28 days.

(4) In ordinary laboratory work there is no apparent advantage in cooling samples in ice or ice water prior to determining their penetration at higher temperatures. Cooling in ice water is therefore not recommended.

(5) Samples should be maintained and tested within 0.1°C . of the desired temperature for accurate work, as a variation in temperature of less than 0.5° in temperature may produce a decided difference in results.

(6) Tests at 4° are not the practical equivalent of properly made tests at 0° .

(7) When making tests at 0° , samples should not be packed in crushed ice, but should be immersed in a brine bath.

(8) The increase in penetration of a material determined under given conditions of temperature and time is, within certain limits, almost proportional to the increase in load. For the 100- and 200-gm. loads variations of as much as 1 gm. do not as a rule seriously affect determinations. It is, however, recommended that in all cases the load should not vary more than 0.2 gm. from that desired.

(9) In any test, proportionally the greatest number of points penetration is obtained during the first second. In the 5-second test approximately one-half of the total penetration is obtained during the first second. A variation of one-half second may, however, produce an appreciable variation in results.

(10) A carefully calibrated metronome is recommended for securing the proper time control.

(11) Aside from possible variations in needles, it is believed that variations in results obtained upon the same material by different laboratories are more probably due to unobserved variations in the methods of preparing the sample and to the control of temperature than to any other causes.

(12) It is believed that a study of the penetration of various types and grades of bituminous materials under a variety of conditions of temperature, load, and time may throw considerable light upon their other physical and chemical characteristics, and may serve as a possible means of identifying their origin and method of manufacture. The writers propose to continue work along this line.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, \$3.00 PER YEAR
v